

[1-¹⁴C]-isopentenyl diphosphate (1 Ci/mol) 25 nmol
 Allylic diphosphate (geranyl diphosphate) 25 nmol
 Potassium phosphate buffer (pH 5.8) 10 mM
 MgCl₂ 5 mM

Enzyme solution 100 µg

H₂O to make 200 µl

After the reaction is over, 200 µl of saturated NaCl was added to the reaction solution and 1 ml of water-saturated butanol was added thereto, which was then agitated, 10 centrifuged, and separated into two phases. To 800 µl of the butanol layer obtained was added 3 ml of a liquid scintillator and then the radioactivity was measured by the scintillation counter. The result is shown in FIG. 2.

The mutant prenyl diphosphate synthase has exhibited a 15 thermo stability which is equal to that of the native geranylgeranyl diphosphate synthase, and is higher than that of the farnesyl diphosphate synthase derived from *Bacillus stearothermophilus*.

The solvent is evaporated from the remainder of the butanol layer by purging nitrogen gas thereto while heating the layer in order to concentrate to a volume of about 0.5 ml. To the concentrate were added 2 ml of methanol and one 5 ml of potato acid phosphatase solution (2 mg/ml potato acid phosphatase, 0.5 M sodium acetate (pH 4.7)) to effect the dephosphorylation reaction at 37° C. Subsequently the dephosphorylated reaction product was extracted with 3 ml of n-pentane.

This was concentrated by evaporating the solvent by purging nitrogen gas thereto, which was then analyzed by TLC (reverse phase TLC plate: LKC18 (Whatman), development solvent: acetone/water=9/1). The developed dephosphorylated reaction product was analyzed by the Bio Image Analyzer BAS2000 (Fuji Photo Film) to determine the location of radioactivity. The result when geranyl diphosphate was used as the allylic substrate is shown in FIG. 3.

The reaction product of the mutant prenyl diphosphate synthase was shown to be a farnesyl diphosphate.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 14

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 330 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Sulfolobus acidocaldarius*
- (B) STRAIN: ATCC 33909

(ix) FEATURE:

- (A) NAME/KEY: Asp-rich domain
- (B) LOCATION: 82-86

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Ser Tyr Phe Asp Asn Tyr Phe Asn Glu Ile Val Asn Ser Val Asn
      5              10              15
Asp Ile Ile Lys Ser Tyr Ile Ser Gly Asp Val Pro Lys Leu Tyr Glu
      20              25              30
Ala Ser Tyr His Leu Phe Thr Ser Gly Gly Lys Arg Leu Arg Pro Leu
      35              40              45
Ile Leu Thr Ile Ser Ser Asp Leu Phe Gly Gly Gln Arg Glu Arg Ala
      50              55              60
Tyr Tyr Ala Gly Ala Ala Ile Glu Val Leu His Thr Phe Thr Leu Val
      65              70              75              80
His Asp Asp Ile Met Asp Gln Asp Asn Ile Arg Arg Gly Leu Pro Thr
      85              90              95
Val His Val Lys Tyr Gly Leu Pro Leu Ala Ile Leu Ala Gly Asp Leu
      100             105             110
Leu His Ala Lys Ala Phe Gln Leu Leu Thr Gln Ala Leu Arg Gly Leu
      115             120             125
Pro Ser Glu Thr Ile Ile Lys Ala Phe Asp Ile Phe Thr Arg Ser Ile
      130             135             140
Ile Ile Ile Ser Glu Gly Gln Ala Val Asp Met Glu Phe Glu Asp Arg

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| 145 | 150 | 155 | 160 |
|---|-----|-----|-----|
| Ile Asp Ile Lys Glu Gln Glu Tyr Leu Asp Met Ile Ser Arg Lys Thr | 165 | 170 | 175 |
| Ala Ala Leu Phe Ser Ala Ser Ser Ser Ile Gly Ala Leu Ile Ala Gly | 180 | 185 | 190 |
| Ala Asn Asp Asn Asp Val Arg Leu Met Ser Asp Phe Gly Thr Asn Leu | 195 | 200 | 205 |
| Gly Ile Ala Phe Gln Ile Val Asp Asp Ile Leu Gly Leu Thr Ala Asp | 210 | 215 | 220 |
| Glu Lys Glu Leu Gly Lys Pro Val Phe Ser Asp Ile Arg Glu Gly Lys | 225 | 230 | 235 |
| Lys Thr Ile Leu Val Ile Lys Thr Leu Glu Leu Cys Lys Glu Asp Glu | 245 | 250 | 255 |
| Lys Lys Ile Val Leu Lys Ala Leu Gly Asn Lys Ser Ala Ser Lys Glu | 260 | 265 | 270 |
| Glu Leu Met Ser Ser Ala Asp Ile Ile Lys Lys Tyr Ser Leu Asp Tyr | 275 | 280 | 285 |
| Ala Tyr Asn Leu Ala Glu Lys Tyr Tyr Lys Asn Ala Ile Asp Ser Leu | 290 | 295 | 300 |
| Asn Gln Val Ser Ser Lys Ser Asp Ile Pro Gly Lys Ala Leu Lys Tyr | 305 | 310 | 315 |
| Leu Ala Glu Phe Thr Ile Arg Arg Arg Lys | 325 | 330 | |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Sulfolobus acidocaldarius*
- (B) STRAIN: ATCC 33909

(ix) FEATURE:

- (A) NAME/KEY: Asp-rich domain coding
- (B) LOCATION: 246-258

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | |
|--|-----|
| ATGAGTTACT TTGACAACTA TTTTAATGAG ATTGTTAATT CTGTAAACGA CATTATTAAG | 60 |
| AGCTATATAT CTGGAGATGT TCCTAAACTA TATGAAGCCT CATATCAITT GTTACATCT | 120 |
| GGAGGTAAGA GGTTAAGACC ATTAATCTTA ACTATATCAT CAGATTTATT CGGAGGACAG | 180 |
| AGAGAAAGAG CTTATTATGC AGGTGCAGCT ATTGAAGTTC TTCATACITT TACGCTTGTG | 240 |
| CATGATGATA TTATGGATCA AGATAATATC AGAAGAGGST TACCCACAGT CCACGTGAAA | 300 |
| TACGGCTTAC CCTTAGCAAT ATTAGCTGGG GATTTACTAC ATGCAAAGGC TTTTCAGCTC | 360 |
| TTAACCCAGG CTCTTAGAGG TTTGCCAAGT GAAACCATAA TTAAGGCITT CGATAITTTTC | 420 |
| ACTCGTTCAA TAATAATTAT ATCCGAAGGA CAGGCAGTAG ATATGGAATT TGAGGACAGA | 480 |
| ATTGATATAA AGGAGCAGGA ATACCTTGAC ATGATCTCAC GTAAGACAGC TGCATTATTC | 540 |
| TCGGCATCCT CAAGTATAGG CGCACTTATT GCTGGTGCTA ATGATAATGA TGTAAGACTG | 600 |
| ATGTCGTGATT TCGGTACGAA TCTAGGTATT GCATTTTCTA TTGTTGACGA TATCTTAGGT | 660 |
| CTAACAGCAG ACGAAAAGGA ACTTGGAAG CCTGTTTTTA GTGATATTAG GGAGGGTAAA | 720 |

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| | |
|---|-----|
| AAGACTATAC TTGTAATAAA AACACTGGAG CTTTGTAAG AGGACGAGAA GAAGATTGTC | 780 |
| CTAAAGGCGT TAGGTAATAA GTCAGCCTCA AAAGAAGAAT TAATGAGCTC AGCAGATATA | 840 |
| ATTAAGAAAT ACTCTTTAGA TTATGCATAC AATTTAGCAG AGAAATATTA TAAAAATGCT | 900 |
| ATAGACTCTT TAAATCAAGT CTCTCTAAG AGTGATATAC CTGGAAAGGC TTAAAAATAT | 960 |
| CTAGCTGAAT TTACGATAAG AAGGAGAAAA TAA | 993 |

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | |
|---|----|
| CATACITTTT TCCTTGTCGCT TGATGATATC ATGGATC | 37 |
|---|----|

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | |
|---|----|
| CATACITTTT TCCTTGTCGCT TGATGATATC ATGGATC | 37 |
|---|----|

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | |
|---|----|
| CATACTTATT TCCTTGTCGCT TGATGATATC ATGGATC | 37 |
|---|----|

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

| | |
|---|----|
| CATACTTATT TCCTTGTCGCT TGATGATATC ATGGATC | 37 |
|---|----|

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

09902641 071201
 T02T00 T020660

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTTCTTCATA CTTATTCGCT TATTCATGAT AGTATT

36

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATTTCATGATG ATCTTCCATC GATGGATCAA GAT

33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTTTCCTTG TGGCTGATGA TATCATG

27

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTTTCCTTG TGCTTGATGA TATCATG

27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TATTTCCCTTG TGCTTGATGA TATCATG

27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TATTTCCCTTG TGGCTGATGA TATCATG

27